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The Role of the Cardioregulatory Nerves in Mediating Heart Rate Responses to Locomotion, Reduced Stroke Volume, and Neurohormones in *Homarus americanus*

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Abstract. Control of decapod crustacean heart activity is believed to be dependent on the regulation of the cardiac ganglion by external input from the central nervous system as well as by circulating neurohormones. This study investigated the roles of these inputs on the heart rates of lobsters exercising on a treadmill. Heart rate increased rapidly at the onset of walking in control animals. This rapid phase was lost after the regulatory nerves were cut, but small increases still occurred. When stroke volume was reduced by cutting alary ligaments, the animals compensated by increasing heart rate; this compensation was lost when the regulatory nerves were cut. In resting animals, injection of serotonin, octopamine, and dopamine induced increases in heart rate. After the regulatory nerves were cut, only dopamine and serotonin injections caused increases in heart rate, suggesting that these amines act on the cardiac ganglion as independent effectors.

Introduction

The neurogenic decapod crustacean heart consists of a single ventricle suspended in the pericardial sinus by an array of alary ligaments. The energy stored as these ligaments are stretched during systole is recovered to re-expand the heart during diastole. The heart fills by means of valved ostia and supplies hemolymph to seven arteries. There is no direct venous supply to the heart.

The basic contraction rhythm of the heart arises from the bursting discharges of the nine-cell cardiac ganglion located on the inner dorsal wall of the heart (Alexandrowicz, 1932). The cardiac ganglion receives extrinsic nerve fibers *via* the paired dorsal nerves that arise from the central nervous system (Alexandrowicz, 1932). Each dorsal nerve contains two accelerator axons and one inhibitory axon. In isolated hearts, stimulation of the accelerator nerves speeds the contraction rate, and stimulation of the inhibitory nerves slows or stops the heart (Maynard, 1953; Florey, 1960; Wilkens and Walker, 1992). *En passant* recordings from the dorsal nerves in semi-intact animals reveal periodic increases in inhibitory nerve firing rates; these increases cause bradycardia (Field and Larimer, 1975; Young, 1978). The role of this autonomic-like control system in regulating heart rate responses in intact animals has not been studied.

Heart rate (f_H) is also modified by all of the neurohormones that have been identified in the pericardial organs (see reviews by Wilkens, 1987; Wilkens and McMahon, 1992). Each of the aminergic neurohormones, dopamine (DA), octopamine (OA) and serotonin (5-HT), trigger tachycardia in isolated hearts (Cooke, 1966; Florey and Rathmayer, 1978; Grega and Sherman, 1975) and in intact animals (Wilkens *et al.*, 1985). In intact animals the possibility cannot be discounted that the neurohormones act indirectly *via* the nervous system as well as directly (Berlind *et al.*, 1970). The experiments reported here were designed to test the hypothesis that f_H in intact lobsters is under continuous modulatory control by cardioregulatory nerves and pericardial aminergic neurohormones.

Materials and Methods

Source and holding condition of lobsters

Homarus americanus of 490–670 g mass were obtained from a commercial supplier and maintained in flowing artificial seawater at 12°C. No differences in performance were observed between males and females.

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Abbreviations: f_H, heart rate; DA, dopamine; OA, octopamine; 5-HT, 5-hydroxytryptamine.

f_H measurement and surgical procedures

Heart beat was measured by electrodes implanted next to the heart in the pericardial sinus. Wires were inserted through holes drilled in the carapace and cemented in place. Signals were amplified (Grass P15 amplifier) and digitized by a Mac Lab/8 and visualized on the Macintosh SE/30 computer running CHART/8 software. In some cases data were also displayed on a Gould RS200 chart recorder.

For surgical interventions, animals were first packed in crushed ice for about 30 min to effect cold "anesthesia." Next, the carapace and hypodermis over the heart were removed. A dam molded from dental impression wax was placed around the opening to prevent hemolymph from spilling over into the bath. The wax was secured to the carapace with cyanoacrylate adhesive. With the heart so exposed, the two dorsal anterior alary ligaments, the dorsal nerves, or both could be cut. The dorsal nerves approach the heart along the dorsal surface of the posterior dorsal alary ligaments and traverse the posterior half of the ventricle in the connective tissue covering the heart before penetrating to the cardiac ganglion. Superficial cuts through the connective tissue over the heart severed these nerves without impairing the alary ligaments. After surgery, the square of carapace was replaced and sealed with melted dental wax and cyanoacrylate adhesive. Sham operations consisted of removing the square of carapace and opening the hypodermis only. Following the return of animals to seawater, f_H usually returned to preoperation rest levels in 2 h; however, tests were not run until the next day.

Induced locomotion

To measure cardiac responses to exercise, lobsters were trained to walk on an underwater treadmill. The treadmill consisted of a cloth-backed sanding belt passed around a motor driven and a tensioning drum. The treadmill chamber was submerged in aerated seawater siphoned from the holding tank and was maintained at 13°–14°C. Some lobsters walked readily from the outset, but others required several training runs before they would exhibit continuous walking. Touching the telson and uropods with a test tube brush was usually an adequate stimulus to induce walking. The treadmill belt traveled at 1.7 $m \cdot min^{-1}$. All observations in this study were carried out in the treadmill chamber. The walls of the chamber were covered with black plastic to isolate the animal from visual stimuli.

Administration of neurohormones

Saline or neurohormones—dopamine (DA), octopamine (OA), and serotonin (5-HT) (Sigma Chemical Co.)—dissolved in lobster saline (Cole, 1941) were injected into the lateral pericardial sinus of resting animals. A syringe pump was used to deliver the injection over a period of 1 min. An Intermedic polyethylene tube (P.E. 160) ran from the syringe to a needle that was inserted into the sinus through a hole that had been drilled into the carapace and covered with dental dam. The injection volume was adjusted to the weight of the animal so that no more than 240 μl would produce a circulating concentration of 1 μM, assuming a 30% hemolymph volume (Gleeson and Zubkoff, 1977). Animals were not handled during the injection and did not appear to be disturbed by the saline injections. They often became agitated, however, during hormone injections. When more than one test was conducted during a day, an average of 4–5 h was allowed for recovery between injections.

Statistical tests

Significance was evaluated with a two-tailed paired Student's t test. A P value < 0.05 was considered significant. Paired t tests were used to compare the heart rates during the last 6 min of the rest period to the first 6 min after the onset of walking or following injections. Examination of the significance of denervation was carried out with an analysis of covariance. In this case the slope of heart rate change, during exercise, in denervated animals was compared to the change seen in intact animals.

Results

Role of dorsal nerves in heart response to startle and exercise

In settled animals, novel stimuli such as shadows, movements in the visual field, and touches to the carapace always triggered periods of bradycardia known as startle responses. These responses diminished when the stimuli were repeated (Fig. 1). Successful cutting of the dorsal nerves was confirmed by the loss of the startle response.

Settled lobsters resting in the treadmill chamber exhibited regular heart rates of 54 ± 13 min^{-1}. At the onset of walking, f_H rapidly increased to 87 ± 5 min^{-1} over the first 2–3 min and then more slowly to 90 ± 5 min^{-1} by the end of the 30-min walk (Fig. 2). While an animal was walking, the swimmerets beat and the abdomen and chelipeds were held slightly elevated and extended. During the 30-min exercise period, animals often stopped and then, when they had drifted back to the bottle brush, accelerated and then resumed a steady pace. The frequency and length of pauses tended to increase with time during the exercise period. At the point of exhaustion, animals would not respond to tactile stimulation. Heart rate and walking behavior were not different between sham-operated and unoperated control animals.

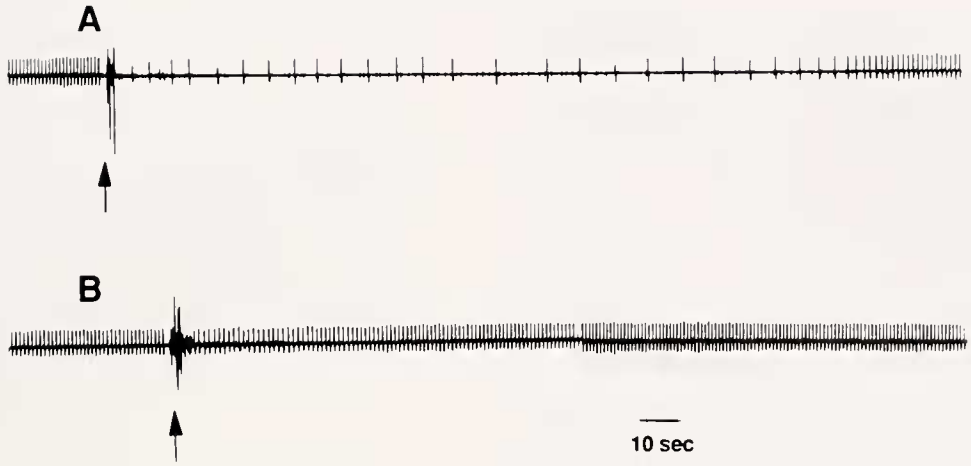

Figure 1. The startle response, a period of bradycardia, seen after passing a shadow (A) across the visual field of a settled lobster. The startle response is usually diminished if the same or a different stimulus is repeated within a few minutes of a first stimulus, in this case tapping the carapace (B). Parts A and B are continuous records.

Following denervation surgery, heart rate returned to previous resting rates in 2–3 h, but it gradually increased over several days thereafter. The data shown in Figure 2 were collected over several days following surgery, and the slightly elevated settled rates reflect this gradual increase. Walking behavior was unchanged from that of controls; however, the rapid phase of tachycardia did not occur. Heart rates did increase slowly from 62 min^{-1} at rest to a maximum of 72 min^{-1}, an increase significantly below that observed in controls. Rates during the last 6 min of walking did not differ significantly from those recorded during the 6-min period prior to walking.

Role of dorsal nerves in response to cardiac impairment

In *Carcinus maenas*, cutting the two dorsal anterior alary ligaments reduced cardiac output of semi-isolated hearts by 25% (Wilkens and McMahon, 1992). One day after these same two ligaments were cut in lobsters, the resting f_H was elevated by 20.6% above controls (Fig. 3).

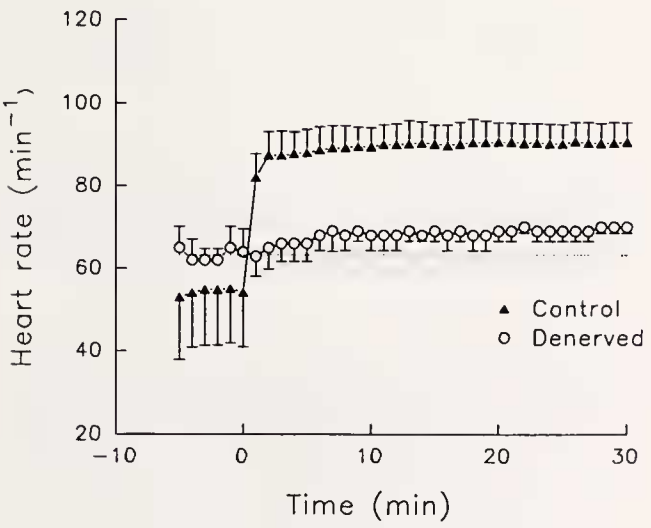

Figure 2. The effects of exercise on the heart rate of intact lobsters (n = 11) and following sectioning of both dorsal cardioregulatory nerves (n = 3). The dotted line is drawn at the mean heart rate of denervated animals for the 6-min period before the start of walking. The treadmill was started at time zero and traveled at 1.7 m · min^{-1}. Mean ± SD.

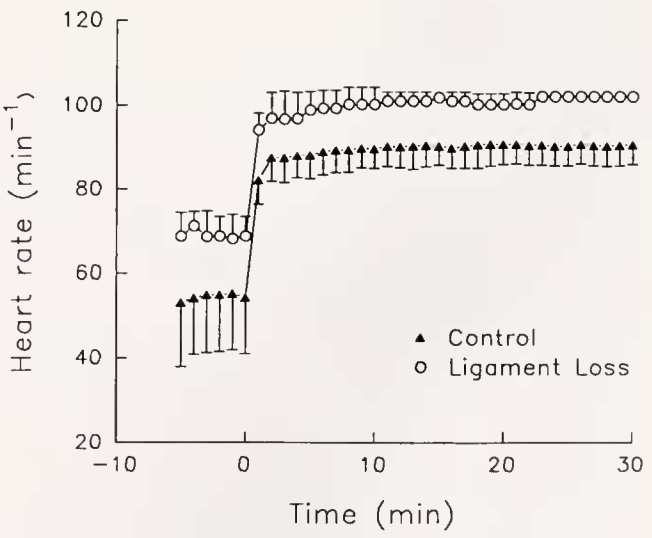

Figure 3. The effect of severing the two dorsal-anterior alary ligaments on heart rate in resting and exercising lobsters (n = 11 for control, n = 5 for operated). The control data are reproduced from Figure 2. The five operated lobsters were taken from that group. The treadmill was started at time zero. Mean ± SD.

During walking, f_H increased on average 11.7% above controls (102 ± 2 min^{-1}). These animals showed no impairment in walking behavior.

On the second or third day after the ligaments were cut, the heart chamber was reopened and the dorsal nerves were sectioned. After this procedure the compensatory increases seen in resting f_H after ligament loss were reversed (Fig. 4). During locomotion, f_H increased slowly and steadily. The final rates after 25 min were significantly lower than those of control or ligament-loss animals. The rates were not significantly different from those observed during the same period in animals with only dorsal nerve loss (Fig. 2). These animals were reluctant to walk and continued to do so only if continuously prodded. Three of the animals refused to walk longer than 21 min. The chelae often dragged along the belt, and after about 10 min of walking the animals seemed to be trying to recruit the chelae to aid walking. The abdomen also dragged.

Responses of heart to aminergic neurohormones

When settled lobsters were injected with enough of each of the aminergic neurohormones to produce a circulating concentration of 1 μM (assuming complete mixing), the result was significant and prolonged tachycardia (Fig. 5), whereas injection of the same volume of saline had no effect. DA caused the largest increase (83 ± 2 min^{-1}), followed by 5-HT (79 ± 2 min^{-1}) and OA (71 ± 3 min^{-1}). Heart rate remained elevated for more than an hour in almost all cases.

The maximum f_H values following injection of DA and 5-HT in denervated settled animals were similar to those

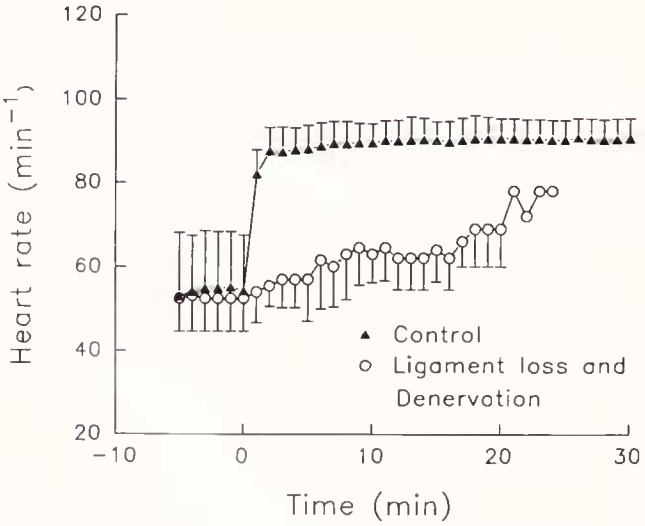

Figure 4. The compounded effects of alary ligament loss and denervation on the heart rate of exercising lobsters (n = 11 for control, same animals illustrated in Figs. 2 and 3; n = 5 for operated, the same denervated animals illustrated in Fig. 3). The treadmill was started at time zero. Mean ± SD.

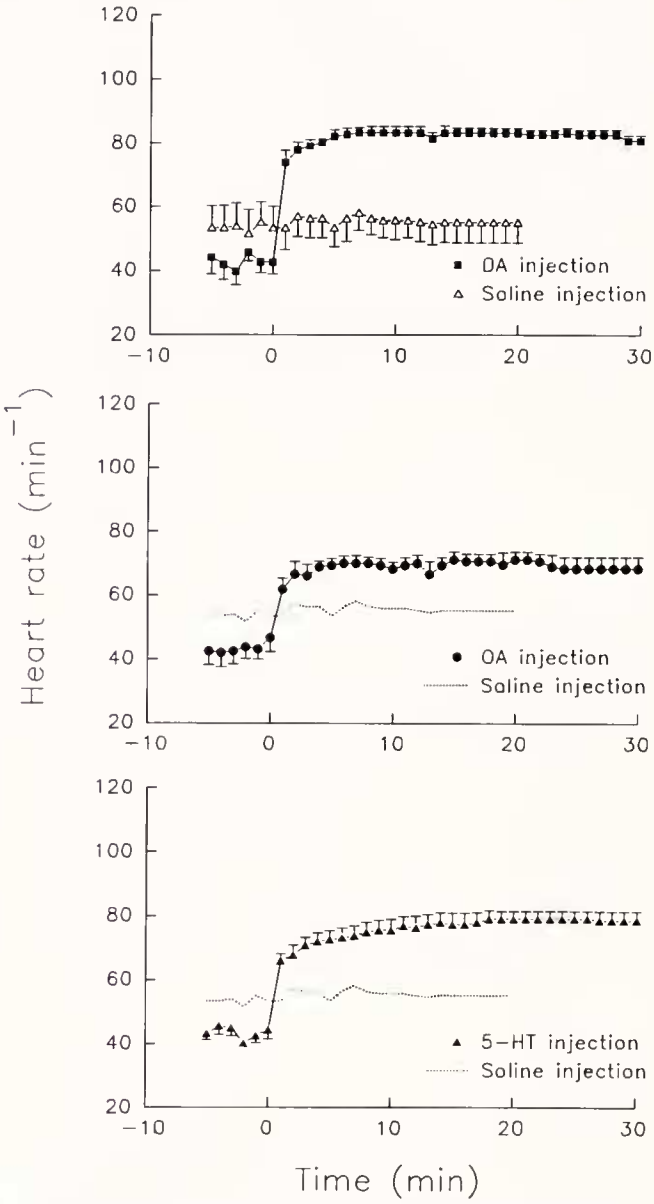

Figure 5. The effects of saline, OA, DA, and 5-HT injections on heart rate of settled lobsters (n = 5). The responses to saline injections are copied in each panel as a reference trace. The amines were presented in random order from animal to animal. Mean ± SEM.

in the control animals (Fig. 6), but they were not maintained at the maximum levels as they were in the controls. All of these animals showed elevated rates at rest compared to controls. OA injections had no effect on f_H; however, the preinjection f_H values were at the same level as in control animals following OA.

Injection of the same amounts of 5-HT into control lobsters just before the treadmill was turned on caused similar increases in f_H, but prevented walking for as long as 15 min. The animals appeared to be stiff and did not

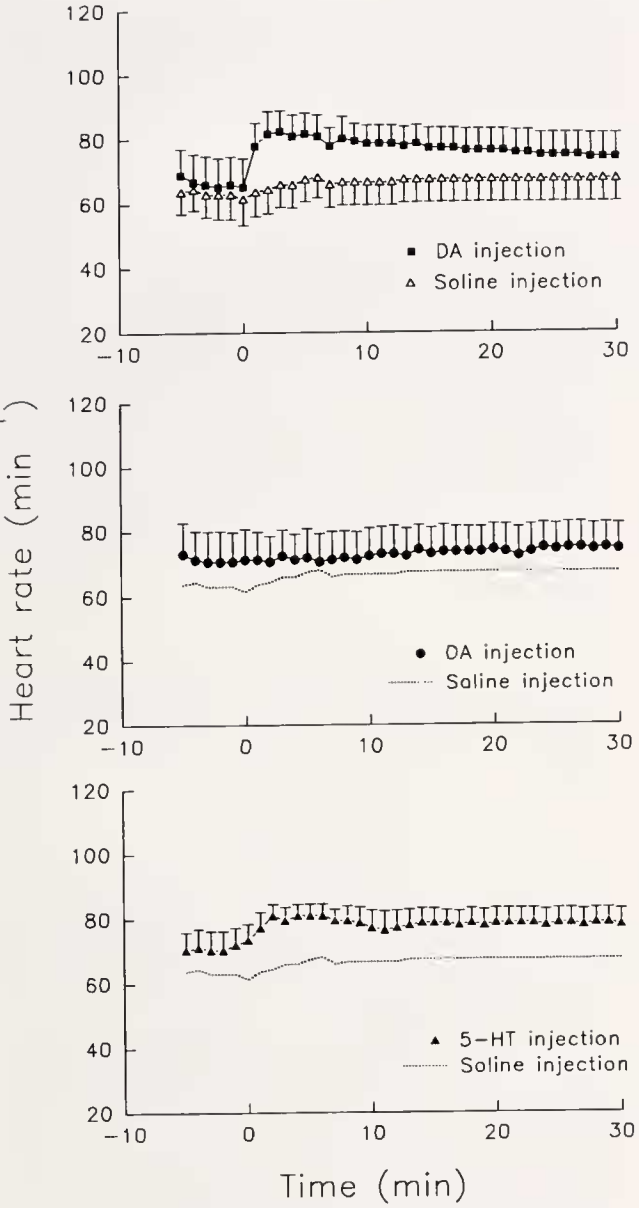

Figure 6. The effects of saline, OA, DA, and 5-HT injections on heart rate in denervated lobsters ($n = 5$). The responses to saline injections are copied in each panel as a reference trace. The amines were presented in random order from animal to animal. Mean ± SEM.

walk even when prodded. Over the next 30 min the willingness to walk gradually increased. DA and OA did not impair walking.

Discussion

Role of dorsal nerves in heart response to exercise

The loss of startle-induced bradycardia after dorsal nerve cutting indicates that the cardioinhibitor nerves are the causative inputs for this behavior. The heart rate response to exercise involves two phases. Phase I is rapid-onset tachycardia, which occurs within the first 2 to 3 min of exercise (Fig. 2). The control system is rather like an on-off switch and does not seem to be graded with duration of exercise. Phase II is a sustained and gradually increasing f_H throughout the period of exercise. The role of the cardioregulatory innervation in these two phases was evaluated following nerve sectioning. In the absence of the dorsal excitatory and inhibitory nerves, the regulation of the heart activity can occur only *via* hemolymph-borne factors. Such factors could include neurohormonal secretions from the pericardial organs lining the pericardial cavity and metabolite end products produced during walking. The absence of Phase I in denervated animals demonstrates the ineffectiveness of these secondary processes in compensating for cardiovascular stress. Therefore, the cardioaccelerator nerves must be responsible for this response, whereas the pericardial neurohormone secretions are not. This finding is consistent with prior *in vitro* investigations, which demonstrated that stimulation of accelerator nerves causes a quick increase in f_H (within 30 s of stimulation; Maynard, 1953; Florey, 1960; Hagiwara, 1961). The small increases in f_H during Phase II of exercising in control, denervated, and dual-operated (ligament loss and denervation) animals may arise from neurohormonal inputs even though the rates never reached control levels. Apparently pericardial secretions alone do not contribute to Phase I, but they may play a role in Phase II. Therefore, Phase II is most likely due to dual action of nervous input and pericardial secretions.

Role of dorsal nerves in response to cardiac impairment

The effects of reduced stroke volume, resulting from ligament loss, were compensated for by an increase in f_H both at rest and while walking. This supports the finding of McGaw *et al.* (1994) that the primary mechanism used to offset the effects of cardiovascular stress is an increase in f_H. The subsequent denervation of these hearts eliminated the elevated resting rates, although the very slow increases in f_H still occurred during exercise. Following the dual insults on cardiac output of ligament loss and denervation, lobsters were reluctant to walk for more than short periods and appeared to fatigue rapidly. These results demonstrate that cardioregulatory innervation plays an important role in primary compensatory cardiovascular responses to exercise.

An examination by McGaw *et al.* (1994) of hemolymph flow patterns during short periods of spontaneous walking showed preferential hemolymph flow to the sternal artery that supplies the periopods and scaphognathites (ventilatory pumps). This redistribution of hemolymph flow is thus another compensatory measure used by animals in the face of cardiovascular stress. Those authors also found

that short-term cardiovascular stress was accompanied by an increase in f_H and stroke volume.

Role of extrinsic nerves in mediating neurohormone action

Injection of any one of the three aminergic neurohormones (OA, DA, and 5-HT) into resting animals brought about a significant increase in the f_H that was maintained for more than 30 min. The circulating half life of these three animals in crayfish and crabs varies from 2 to 13 min (Hoeger and Florey, 1989; Hoeger, 1990; Bininda *et al.*, 1992). These clearance studies were performed at temperatures similar to those used during the present study. Clearance rates in lobsters have not been determined, but if we can assume that they are similar to those in crayfish and crabs, then the duration of the elevation in f_H is greater than the expected residence time of the hormones. In denervated animals, only DA and 5-HT produced short-term increases in resting f_H; OA injection produced no change. The initial increase in f_H brought about by DA and 5-HT injection lasted for only about 10 min and gradually declined thereafter. The initial responses may have arisen from the period when circulating amines were highest, while the decline thereafter may indicate that the dorsal nerves play a role in maintaining neurohormone action beyond the expected amine residence time. In other words, the prolonged elevation of f_H in intact animals may arise from long-term increases in cardioaccelerator or decreases in cardioinhibitor tonic firing rates. The exogenously applied DA may be mimicking the action of the cardioaccelerator nerves, because recent evidence suggests that DA may be the neurotransmitter released by the cardioaccelerator nerves (Yazawa and Kuwasawa, 1992, 1994).

Effect of neurohormones on posture and walking

The injection of 5-HT and octopamine into lobsters induces stereotypical postures thought to resemble those seen in dominant (5-HT) and subordinate (OA) animals (Livingstone *et al.*, 1980; Kravitz, 1990). 5-HT caused animals to stand tall on the tips of their walking legs with their chelae extended and open and their abdomens flexed. OA injections caused the animals to stand low to the substrate with their claws and walking legs extended and pointing forward and with their abdomens hyperextended upward. In our experiments, lobsters injected with similar doses of 5-HT also assumed the expected stance, but were quite stiff and unable to walk for up to 15 min. They slowly regained ability to walk over the following 30 min. In *Carcinus*, 5-HT triggers a rigid posture and reduces an animal's ability to right itself when placed on its back (McPhee and Wilkens, 1989). Perhaps it is inappropriate to suggest that the 5-HT–induced posture mimics the dominant stance displayed by untreated lobsters in natural settings, because these animals are unable to move. On the other hand, the ability of lobsters to walk was unaffected by OA injections.

Conclusions

The focus of this investigation was to determine the role of cardioregulatory nerves in regulating the f_H of *H. americanus*. Our data support the conclusions that the cardioinhibitor nerves are responsible for startle-induced bradycardia and that the cardioaccelerator nerves cause the rapid-onset tachycardia associated with walking. The continued gradually increasing heart rate during long walks may be partially accounted for by hemolymph-borne factors including neurohormones. The cardioaccelerator nerves are also responsible for the compensatory increases in f_H in animals whose stroke volume was artificially reduced.

Acknowledgments

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